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Microplastics in wastewater treatment plants in South-Eastern Norway: detection and critical assessment of methodology

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This thesis is worth 60 study points

Abstract

Microplastic has gained increasing attention since it is an environmental concern and it has been found in water, soil and air. Wastewater treatment plants have been reported as pathway for microplastics to aquatic environments and soil, even though the efficiency of removal of microplastics from wastewater treatment plants has been shown to be high in some studies. This study aims to investigate the presence of microplastics in four wastewater treatment plants in Norway (both in wastewater and sludge samples) and also to do a critical assessment of the methodology used. The results showed that fibers/filaments was the most common category. In overall, microplastic particles from wastewater samples consisted of fibers/filaments (71.5%), fragments (8.9%), other shapes (8.9%) and beads (6.8%). For the sludge samples, Fibers/filaments consisted 66.2%, fragments 30.9%, other shapes 1.8% and beads 1.2%. Black fibers were the most common. The mean of total microplastic particles calculated for the facilities varied from 289 to 829 (MP/L) in the influent samples. In the effluent samples, it varied from 144 to 746 MP/L. In sludge samples, the mean of total microplastic particles for the treatment plants varied from 13770 to 37502 MP/Kg (d.w). However, these results should be analysed with caution since the samples were not subjected to chemical analysis, only visual identification. It is possible that the presence of non-plastic particles resulted in an overestimation; or underestimation due to barely visible particles and the difficult identification of so small particles. There was a significant difference between the total numbers of microplastic particles found for some facilities. However, more studies should be done to understand why this difference exist, since many factors are involved. There was no significant difference between months and total microplastic particles found. No significant correlation was found between population equivalent and total microplastic particles in the influent samples. Regarding methodology, many topics were discussed from the extraction to identification in order to contribute with information to develop a standardized methodology in future studies.

Contents

Acknowledgement

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Bø i Telemark, 08/05/2019

Izabella Lage

1 Introduction

Plastic has become a widely used material in various types of products because it is cheap, lightweight and durable. However, in the last 30 years, scientists have realized that these same characteristics make it harmful to our environment. The reason is that plastic does not biodegrade in nature so it is very difficult to eliminate it (Shaw and Sahni 2014, Sigler 2014). Recently, a kind of plastic has gained increasing attention: Microplastics.

Microplastics can be defined as plastic that are < 5mm in size and can be divided in two types: primary microplastics, which has been manufactured to be of this small size; and secondary microplastics, which are produced from the breakdown of macroplastics (Murphy et al. 2016). Examples of microplastics include fibers from clothes made with synthetic materials; microbeads from personal care products; and also fragments from breakdown of larger plastics (Fendall and Sewell 2009, Browne et al. 2011). Experiments show that in a single wash, a domestic washing machine can produce more than 1900 fibers from a unique garment (Browne et al. 2011).

Microplastic pollution is a growing globally issue that can be found in water, soil and air (Ou and Zeng 2018). It can cause problems for wildlife because it can be accidentally ingested as it can be mistaken for food (Wright et al. 2013). Due to the small size, microplastics may be more bioavailable to lower trophic levels, and trophic level transfer has been shown in mussels and crabs (Farrell and Nelson 2013). In addition, microplastics may contain heavy metal, polychlorinated biphenyls (PCBs) and hydrophobic persistent organic pollutants (POPs), which have a greater affinity with the hydrophobic surface of the plastic compared with seawater. Microplastics have a large surface area to volume ratio causing them to adsorb different pollutants (Mato et al. 2001, Hirai et al. 2011).

Microplastics have been found in influent, effluent and sludge of wastewater treatment plants (WWTPs) (Magnusson and Norén 2014, Bayo et al. 2016, Murphy et al. 2016, Ziajahromi et al. 2017). Wastewater from households (including bathroom, toilet and kitchen), institutions, commercial shops, industries and sometimes rainwater run-off end up in the wastewater treatment plants (Magnusson and Norén 2014).

Since these small plastic particles from various types of sources appear to be transported with the wastewater and through sewage treatment, consequently, this may end up in the aquatic environment (Browne et al. 2007). According to Magnusson and Norén (2014), Wastewater treatment plants (WWTPs) have been pointed out as important entrance route to the marine environment for microplastics and other types of anthropogenic particles. Although the efficiency of removal of microplastics from wastewater treatment plant has been shown to be high in some studies (Magnusson and Norén 2014, Lares et al. 2018), microplastics continue to be released in the recipient waters every day (Lares et al. 2018).

Some studies suggest that the majority of microplastics may be retained in the sludge (Magnusson and Norén 2014, Talvitie et al. 2017) .Thus, It can also be an entrance route to the environment, through the soil, since sludge is used in agriculture as fertilizers and on green constructions (Bayo et al. 2016, Talvitie et al. 2017). Lusher et al. (2017) found microplastics in all sludge samples from eight wastewater treatment plants in Norway.

However, the lack of standardized and applicable methodology of microplastic sampling and identification in organic-rich samples has limited the evaluation and can lead to incorrect estimation of it (Ziajahromi et al. 2017). Besides that, the non-existence of standardized method for sampling hampers the comparison between different studies (Talvitie et al. 2015). According to Lares et al. (2018), in the field of microplastic research, there are high amount of uncertainties during the steps of sampling, sampling treatment and identification. Standardized protocols must be developed in order to promote reliable results.

In this context, this study aims to investigate the presence of microplastic in the influent and effluent wastewater, as well as in the sludge of four wastewater treatment plants in Norway; to do a critical assessment of the methodology used in this study to extract and identify microplastics. Since it is a relative new topic which needs to be improved in the future, observations found in this study may contribute somehow to the development of future procedures.

2 Methods

2.1 Wastewater treatment plants

This study was conducted from June to October 2018 at four wastewater treatment plants located in south-eastern Norway, in Telemark County.

The location and name of each wastewater treatment plant were anonymous preserved and in this way, it was named A, B, C and D for this study.

A brief description of each facility and the dates of sampling are detailed below (Table 2-1 and 2-2).

Table 2-1 Description of wastewater treatment and sludge phase analyzed in this study in each Wastewater Treatment Plant.

	Wastewater Treatment Plant A	Wastewater Treatment Plant B	Wastewater Treatment Plant C	Wastewater Treatment Plant D
Type of Treatment	Screening, sand/fat removal, chemical dosing and sedimentation.	Screening, sand/fat removal, pre-sedimentation, chemical dosing and post sedimentation.	Screening, sand/fat removal, chemical dosing and sedimentation.	Screening, sand/fat removal, chemical dosing and flotation.
Phase of Sludge analyzed in this study	Treated (Anaerobic treatment process)	Raw dewatered sludge	Raw dewatered sludge	---

Table 2-2 Sampling period in each wastewater treatment plant.

	Wastewater Treatment Plant A	Wastewater Treatment Plant B	Wastewater Treatment Plant C	Wastewater Treatment Plant D
Date of Wastewater sampling	15-16 June18* 09-10 July 18 19-20 Aug 18 15-16 Sept 18 11-12 Oct 18*	15-16 June 18* 9-10 July 2018* 19-20 Aug 18* 15-16 Sept2018 11-12 Oct 2018	16 June 18* 9-10 July 18* 19-20 Aug 18* 15-16 Sept 18* 18-19 Oct 18*	15-16 June 18 9-10 July 18* 19-20 Aug 18* 15-16 Sept 18 11-12 Oct 18*
Date of Sludge sampling	15-16 June 18 09 July 2018 19-20 Aug 18 15-16 Sept 18 12 October 18	16 June 2018 10 July 2018 ? August 2018 16 Sept 2018 12 October 18	16 June 2018 10 July 2018 20 July 2018 15 Sept 2018 19 October 18	-----

*Some samples received were dated with only one day. When this was the case, it was assumed that the mentioned date was from the last day of the sampling, the previous day was added to obtain the collection period.

2.2 Sampling

The samples were collected by the staff of each wastewater treatment plant. Samples were taken once a month: one sample of wastewater influent, one sample of wastewater effluent and one sludge sample (Table 2-2). Different methodologies were used for wastewater and sludge sampling since sludge samples contain more solid material than wastewater.

Regarding the wastewater sampling, each facility received the same instructions on how this should be done. The sampling of the influent and effluent wastewater was performed through the automated sampling device which usually collects sample for routine chemical analysis of the facility. The samples were collected by the machine over a period of 24 hours and then mixed manually in the container. A subsample was taken and placed in an aluminum can and frozen.

Depending on the size and structure of wastewater treatment plant, it could have two automated sampling devices instead of one to collect the effluent samples. The wastewater that arrives at the station is divided into two equal substations. The subsample was collected by a certain volume from each machine and mixed in a single metal can. Each metal can was identified with name of the facility, date, and the 24h wastewater volume of flow. The samples were frozen.

The sludge samples were also collected once a month by the staff of each facility. The samples were taken from the sludge container and placed in aluminum can and frozen. However in the facility D, it was collected in an earlier stage of the treatment process, where sludge was still mixed with water. Thus, the sample that was contained a lot of water mixed with sludge. Because of this situation, it was decided not to use these samples.

2.3 Extraction and Analysis

Since there is no standardized methodology, the procedures below were developed after many previous tests with the samples based on different scientific papers (Mohamed Nor and Obbard 2014, Murphy et al. 2016, Horton et al. 2017, Lusher et al. 2017, Tagg et al. 2017, Frias et al. 2018). However, many adjustments and modifications were necessary. These procedures were developed in cooperation with another USN`s master student.

2.3.1 Wastewater samples

2.3.1.1 Preparation and Filtration

Each sample was taken from the freezer one day before the day of the extraction. After the sample was melted, the metal can was shaken for 60 seconds with the lid on. The lid was taken off and the can was stirred with a glass pin for 30 seconds. 100ml of the wastewater was poured into 500 ml cylindrical glass beakers with a funnel on the top. For safety reasons, the samples were added to 200 ml of 70% ethanol (C_2H_5OH) and stored for 15 minutes in order to kill pathogenic bacteria.

The solution was mixed and vacuum filtrated using a Whatman GF/C glass microfiber filter (Diameter 47mm – pore size 1,2µm) (Horton et al. 2017). If more than one filter was required, the filtered volume was noted.

The remaining content in the cylindrical beaker and glass filter was washed with distilled water into the vacuum filter (to get adhered particles). A new GF/C filter was used for this process. The filters were put in petri dishes covered by aluminum foil. The petri dishes were identified by name of the facility, date and type of sample (Influent or Effluent).

Approximately seven drops (sufficient amount to wet the whole sample) of 30% Hydrogen peroxide (H_2O_2) was added and stored in the heating oven till its dry. The oven temperature was always bellow 60º C to prevent plastic melting. According to Munno et al. (2018), temperature should not be higher than 60º C to minimize the loss of microplastics, specially microbeads from personal care products.

These steps were applied to all samples except for June samples from the facility A. It was the first sample that the methodology was tested and modified thereafter. The only difference from these June samples to the others was that the whole sample was used instead of only 100 ml. Only the first two filters from June of facility A sample were analyzed totaling 133 ml.

2.3.1.2 Identification

Counting and characterization was performed in the Stereo microscope (Zeiss, Discovery V.20). The filter was placed on a glass petri dish containing two black lines of cotton forming a cross. In this way, the filter was divided by two diagonally lines (Figure 2-1).

For the analysis of the filters, a quick overlook was made first for counting the larger microplastic particles. After that, 15 areas of each line (Field of view 2.5mm: 90.2x to 93.8x magnification) were analyzed. A total of 20 areas between the diagonal lines were also analyzed (five areas in each quadrant) (Figure 2-1).

The filters used for cleaning the cylindrical beaker and vacuum filter were analyzed by overview of the entire filter for a maximum of 20 minutes.

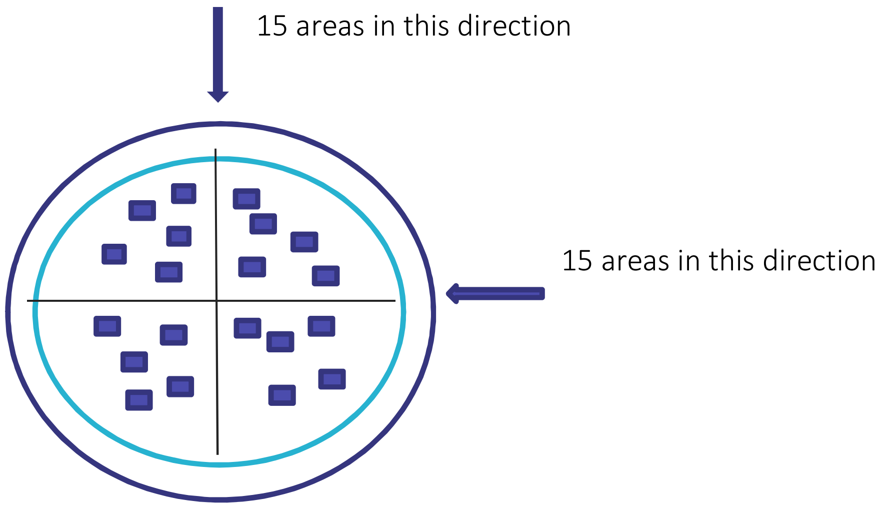

Figure 2-1 Illustration of a filter with the two diagonally cotton lines plus the five random areas in each quadrant used for microplastic counting and characterization.

In the results found, the following formula was used to obtain the number of microplastic particles (MP) per 100 ml:

$$\frac{MP}{100ml} = \frac{\text{filtration circle area}}{\text{analyzed area x 50}} \text{x MP} \qquad (2\text{-}1)$$

Filtration circle area = 962mm²

Diameter filtrated area of filter = 3.5 cm

Analyzed area = 5.54mm²

Total number of analyzed areas = 50

Then this result from the square was added to the amount of microplastic particles found during the quick overlook. Particles found on the filter used to clean the remaining content from the cylindrical beaker and vacuum filter was also added.

The microplastic particles found were classified in the following categories: fibers and filaments; fragments; beads and other shapes. Particles that could not be defined were framed as unknown. The particles were also categorized by color: white/transparent, blue, red, black, green and other colors (Frias et al. 2018).

In order to reduce misidentifying of particles, Mohamed Nor and Obbard (2014) established some criteria to identify microplastics as: no cellular or organic structures are visible; colored particles are homogenously colored; fibers are not segmented. Horton et al. (2017) included more characteristics and the particles should meet at least two of these criteria: unnaturally colored compared to the majority of other particles in the sample and appear to be a homogenous material or texture; unnaturally brightly colored coating on another particle; unnatural shape; fiber that remained intact with poke with tweezers; shiny/glassy; can be compressed without being brittle. Due to particles size and the type of sample (rich in organic material), it was not possible to follow all these criteria as will be explained later in the results and discussion.

Lenz et al. (2015) suggests that using only visual identification based on morphological criteria leads to misidentification of a significant amount of particles. However, some authors have used only visual identification in their studies (Dris et al. 2015, Estahbanati and Fahrenfeld 2016, Michielssen et al. 2016, Peters and Bratton 2016, Sutton et al. 2016). Due to the high cost of laboratory chemical analysis, it was not possible to use additional methods of analysis in this study, such as Fourier-transform infrared spectroscopy (FTIR) or Raman spectroscopy.

2.3.1.3 Statistical Analysis

All statistical tests were performed using R Commander version 2.5-1. One-way ANOVA test was used to assess differences in the abundance of microplastic particles per facility and per month. If there was a significant difference, post–hoc analysis was done with Tukey`s HSD, to see what the differences were. Chi-square goodness of fit test was used to confirm the most abundant category/color. Spearman´s rank correlation was used to analyze if there was a correlation between the population equivalent of each facility and the total microplastic particles found in the influent samples. Also, to check if there was a significant correlation between the 24h volume flow and total microplastic found in influent samples.

2.3.2 Sludge samples

The methodology below was based on Horton et al. (2017), Lusher et al. (2017), Tagg et al. (2017) with some modifications.

2.3.2.1 Pre-treatment

It is known that extracting microplastics from sludge samples is very challenging due to the complexity and high amount of organic matter mixed with microplastics (Sujathan et al. 2017).

The samples were taken from the freezer one day before the extraction. The melted sample was mixed thoroughly 1 minute. Three subsamples of 10g each from different areas (top, middle and bottom of the container) were collected. These subsamples were mixed for 1 minute and 10 g (w.w) of subsample was taken out into a 250ml beaker. For safety reasons (to kill pathogenic bacteria), 20 ml of 70% ethanol was added in the 10g subsample and waited for 15 minutes. Then, the beaker with an aluminum lid was put in another pan with water, over a heating plate at maximum 60º C to evaporate the ethanol.

After this step, the beaker with the subsample was put in a heat oven until it was dry. The temperature of the oven was below 60º C to avoid plastic melting. The dry weight sludge was measured.

Fenton´s reagent, a mixture of 20 ml of 30% hydrogen peroxide and 0.0667g of Iron (II) sulfate heptahydrate ($FeSO_4*7H_2O$)/10 ml of distilled water was added (Concentration 6.67mg/ml). The sample was stored for two-three hours in room temperature to degrade most of the organic matter. According to Lusher et al. (2017), Fenton´s reagent is a successful, cost and time-effective method for reducing large quantities of organic material in sludge samples.

2.3.2.2 Freshwater flotation

In the same beaker, “freshwater” (vacuum filtrated tap water) was added until 1 cm below the brim. The solution was mixed for 30 seconds to make it homogeneous. Then it was left to settle for one hour in a larger vessel with an aluminum foil as a lid. “Freshwater” was poured to the beaker allowing overflow to the larger vessel. The overflowed top layer was filtrated through Whatman GF/C glass microfiber filter

(Diameter 47mm). The middle layer was filtrated through another filter. This step was important to take out the water from the bottom sample to start the flotation step with Zinc chloride ($ZnCl_2$).

It is important to note that there were different types of sludge samples and for this reason the flotation step did not work for some of them as will be explained in the results and discussion section. For samples that flotation did not work, after filtering the overflow of freshwater flotation, the remaining sample was mixed and filtered into new filters, and thus the flotation step by using Zinc chloride was not performed.

2.3.2.3 Zinc chloride flotation

Zinc chloride (Concentration 1.8g/cm³) was added to the same beaker until 1 cm below the brim. The solution was mixed for 45 seconds till it became homogeneous. This beaker was put back into the larger vessel and covered with aluminum foil for two hours. Then, additional Zinc chloride was added allowing overflow of the top layer into the larger vessel. The overflow layer was filtrated. This filter was flushed with distilled water to collect floated particles and remove Zinc chloride.

The rest of the sample was mixed for 15 seconds and vacuum filtrated using another filters. These filters were also flushed with distilled water.

The filters were stored in a petri dish and put in a heat oven until it was dry. As in the wastewater procedure, the oven temperature was always bellow 60º C to prevent plastic melting.

2.3.2.4 Identification and Statistical analysis

The filters were then analyzed by using Stereo microscope (Zeiss, Discovery V.20) for maximum 15 minutes each (Field of view 6.5mm: 35.2x to 35.6x magnification). The microplastic particles were categorized in the same way as for the wastewater samples.

Regarding the statistical analysis, it was done as explained in chapter 2.3.1.3 Statistical analysis to assess differences in the abundance of microplastic particles per facility and per month and to confirm the most abundant category/color.

2.4 Contamination Control

The actions below were performed in order to reduce and control sample contamination during the extraction and analysis step (wastewater and sludge procedure):

- Equipment such as beakers, cylinder beakers, vacuum filter and others were cleaned three times with distilled water.

- All work surfaces (work table and fume hood) were cleaned with ethanol 70% before the beginning of work.

- Plastic Petri dishes used to store the filters at the extraction step were covered by aluminum foil.

- Clean filters in petri dishes were left out during the whole day of work to capture microplastic particles from air (One at work table and one at the fume hood). These filters were analyzed in the stereo microscope later. The steps above was based on Murphy et al. (2016) with some modifications.

3 Results

For a better understanding, the results will be presented in tree main topics: wastewater samples, sludge samples and methodology main observations.

3.1 Wastewater Samples

3.1.1 Categories and colors

71.5% of the microplastic particles were included as fibers/filaments; 12.8% as fragments; 8.9% as other shape´s category and 6.8% as beads (Figure 3-1). Excluding the unknown category, Fibers/Filaments were more abundant than the other categories in overall (Chi-squared goodness of fit test: x-squared=25573, df=4; p-value < 0.001).The particles found were very small which made the visual identification a challenge. This generated a large number of particles framed as unknown during the visual identification (Figure 3-2).

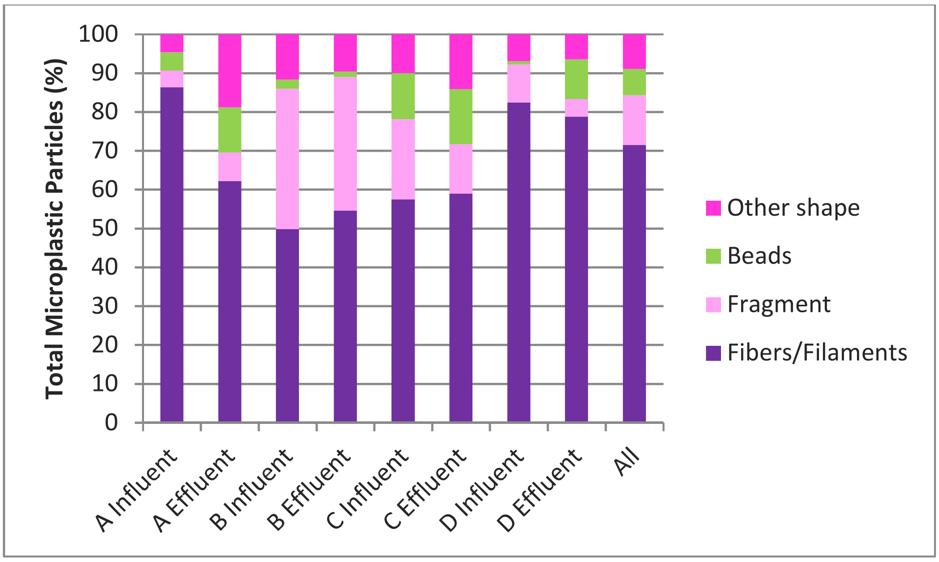

Figure 3-1: Percentage (%) of total microplastic particles found in influent and effluent samples of each facility during the study period from June to October 2018 (Percentage based on the calculated result of microplastic particles per liter).

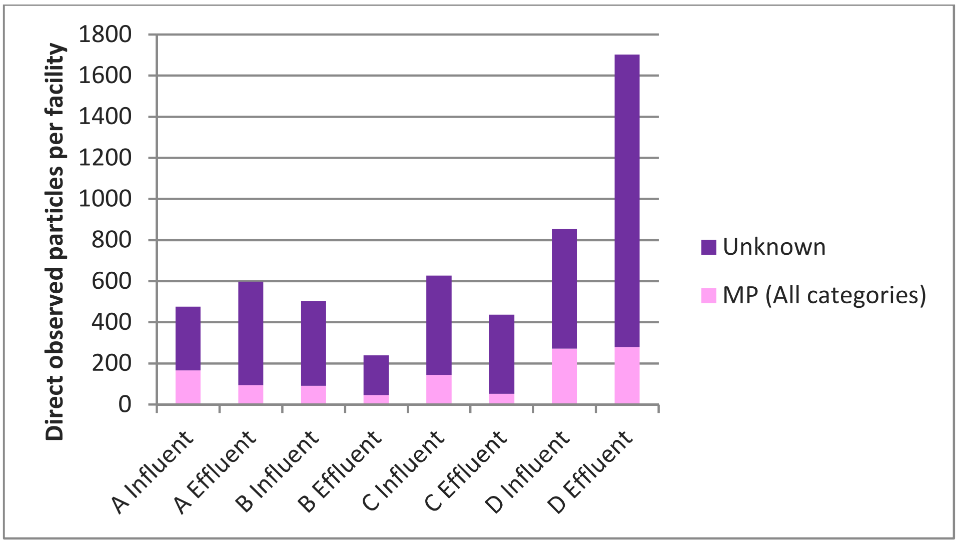

Figure 3-2: Total of directly counted microplastic particles (D.C) during visual identification framed as Unknown compared to the amount of total microplastic particles found in influent and effluent samples during the study period from June to October 2018 in each wastewater treatment plant.

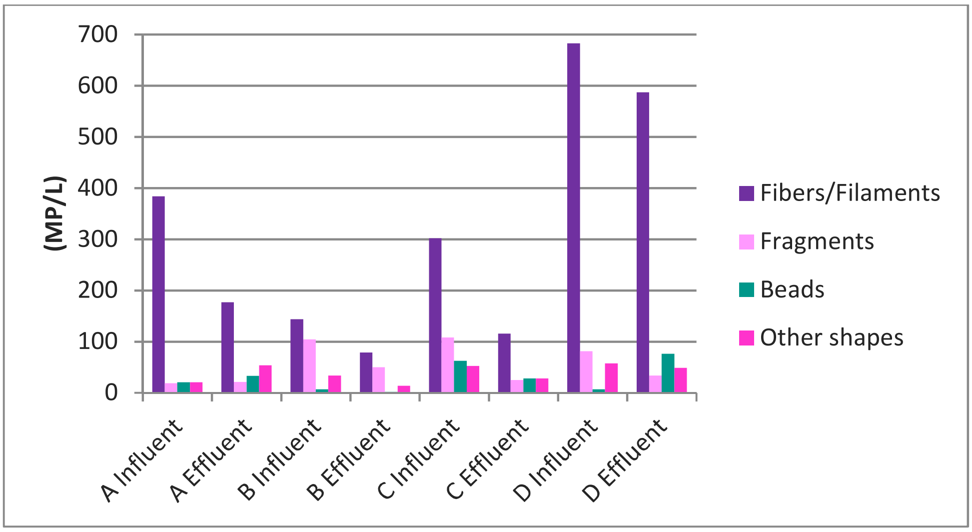

Figure 3-3 Mean of the total numbers of microplastic particles per liter (MP/L) found in influent and effluent samples of each category and facility during the study period from June to October 2018.

The mean of total microplastic particles found in each facility were between 289 (Facility B) to 829 (Facility D) (MP/L) in influent samples. In effluent samples, It was between 144 (Facility B) to 746 (Facility D) microplastic particles per liter (Table 3-1).

Table 3-1 Mean of directly counted microplastic particles (D.C) and of calculated numbers of microplastic particles per liter (MP/L) in influent and effluent samples from each facility during the study period from June to October of 2018.

	Fibers/Filament		Fragments		Beads		Other shapes		Total MP Particles	
Facility	D.C	1L	D.C	1L	D.C	1L	D.C	1L	D.C	1L
A Influent	30*	384	1	19	1	21	0	21	33	445
A Effluent	15*	177	1	21	1	33	2	54	19	285
B Influent	12	144	4	104	0	7	1	34	18	289
B Effluent	6	79	2	50	0	2	0	14	9	144
C Influent	21	302	4	108	2	63	2	53	29	525
C Effluent	8	116	1	25	1	28	1	28	10	196
D Influent	49	683	3	81	0	7	2	58	54	829
D Effluent	51	587	1	34	2	76	1	49	56	746

*June data was extracted from 133 ml of wastewater.

The most abundant color of fiber/filaments was black (64.7%) (Chi-squared goodness of fit test: x-squared=19953; df=5; p-value <0.001). Blue color constituted 14.1%, Red color 8.3%, White and transparent 6.2%, Other Color 5.5% and green color 1.2% (Figure 3-4). White/Transparent fibers may have been underestimated.

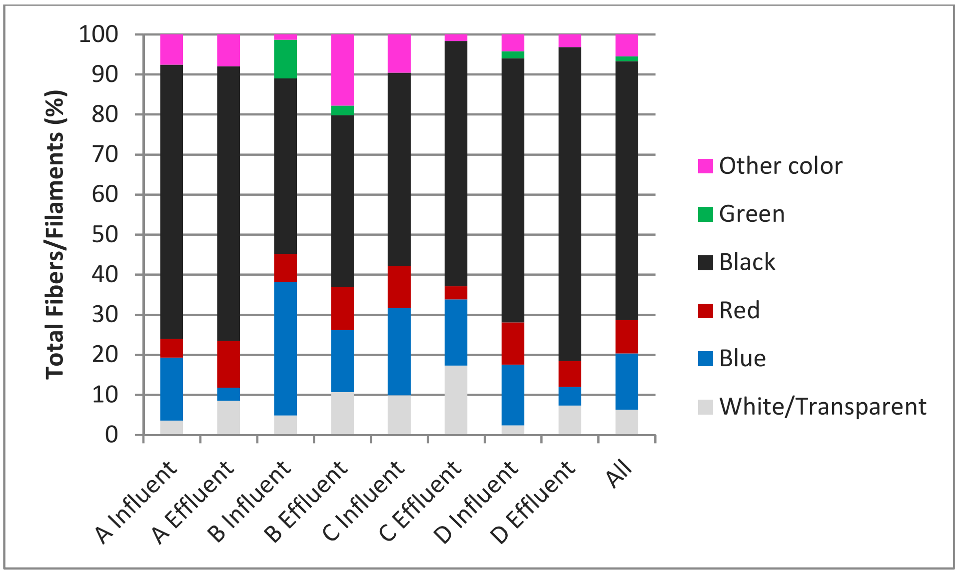

Figure 3-4 Color composition (%) of Fiber/Filaments found in influent and effluent samples of each wastewater treatment plant during the period from June to October 2018 (Percentage based on the calculated result of microplastic particles per liter).

3.1.2 Differences between facilities

There was a significant difference in the total amount of microplastic particles found between the facilities (ANOVA: F=3.615; df=3 p-value = 0.036). However, the difference was only between facility D and B in the influent samples (Tukey HSD: D-B p-value =0.026). The influent samples of facility D had the highest number of total microplastic particles (Mean 829 MP/L ±483) and facility B had the lowest number of microplastic particles (Mean 289 MP/L ± 25). An exceptionally high number of microplastic particles (MP/L) were observed for facility D in the month of October (1641 MP/L) (Figure 3-5).

Regarding the effluent samples, the ANOVA test also showed that there was a significant difference in the total amount of microplastic particles found between the four facilities (ANOVA: F value= 12.79; df=3; p-value < 0.001). However, the difference was between facility D and the other facilities (Tukey HSD: D-B: p-value <0.001; D-C: p-value <0.001; D-A: p-value = 0.003). The effluent samples of facility D had also the highest number of total microplastic particles (Mean 746 MP/L ±305) (Figure 3-6). Although, facility B had the lowest mean of total microplastic particles (Mean 144 MP/L ±69), Tukey HSD did not show significant difference between facilities A, B and C (Tukey HSD: C-B: p-value 0.964; A-B: p-value=0.581; A-C: p-value=0.845).

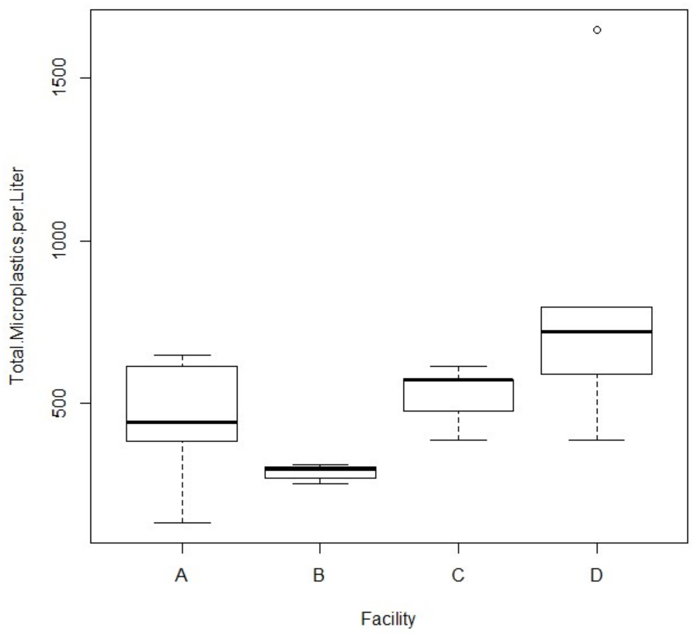

Figure 3-5 Total Amount of microplastic particles per Liter (MP/L), in influent samples, from each wastewater treatment plant during the study period from June to October 2018.

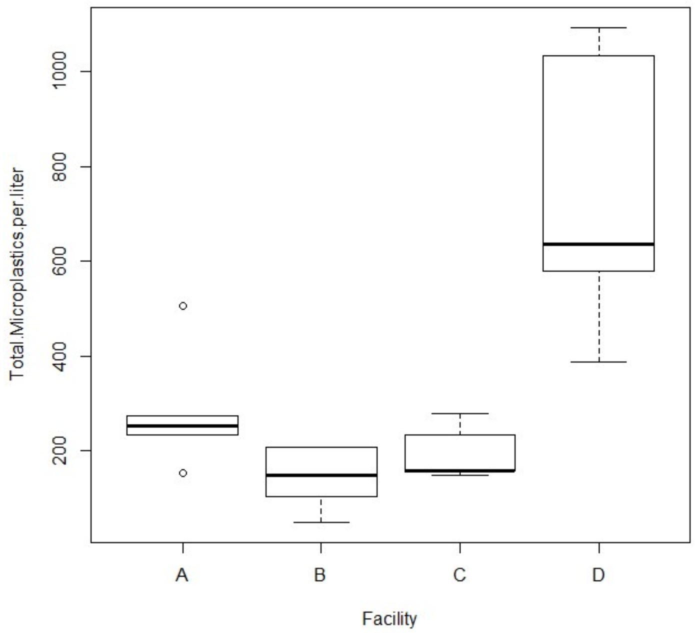

Figure 3-6 Total Amount of microplastic particles per Liter (MP/L), in effluent samples, from each wastewater treatment plant during the period from June to October of 2018.

There was no significant difference in the total amount of microplastic particles found between the months in the influent samples (ANOVA: F= 0.967; df=4; p-value= 0.454) or in the effluent samples (ANOVA: F=0.411; df=4; p-value = 0.798)

No significant correlation was found between the population equivalent of wastewater treatment plants and total microplastics found in the influent samples (Spearman`s: S=762.46; p-value= 0.061). There was also no significant correlation between 24h volume flow of each sampling date and the total microplastic particles found in the influent samples (Spearman`s: S= 1467.1; p-value= 0.666).

3.2 Sludge samples

3.2.1 Categories and colors

Microplastic particles from sludge consisted of Fibers/Filaments (66.2%), Fragments (30.9%), other shapes (1.8%) and Beads (1.2%) (Figure 3-7). At facility A, 50.3% of the microplastic particles were Fragments and 46.2% of Fiber/Filaments. In Facility A, a large amount of transparent film type was found and included in the fragment category.

The mean of total amount of microplastic particles found varied between 13770 (Facility B) and 37502 (Facility A) microplastic particles per kilo (d.w) (Table 3-2).

Table 3-2 Mean of the total amount of directly counted microplastic particles (D.C) during the visual identification and Mean of calculated microplastic particles per kilo sludge (d.w) of each facility, during the period from June to October 2018.

	Filament/Fiber		Fragment		Beads		Other Shape		Total	
Facility	D.C	MP/Kg (d.w)	D.C	MP/Kg (d.w)	D.C	MP/Kg (d.w)	D.C	MP/Kg (d.w)	D.C	MP/Kg (d.w)
A	65*	17329	70	18882	3	707	2	585	140	37502
B	36*	12751	2	703	0	70	1	247	39	13770
C	39*	13382	2	714	0	0	1	322	42	14419

*Directly counted particles in a total of (d.w): A= 18.59g; B= 14.07g; C= 14.52g

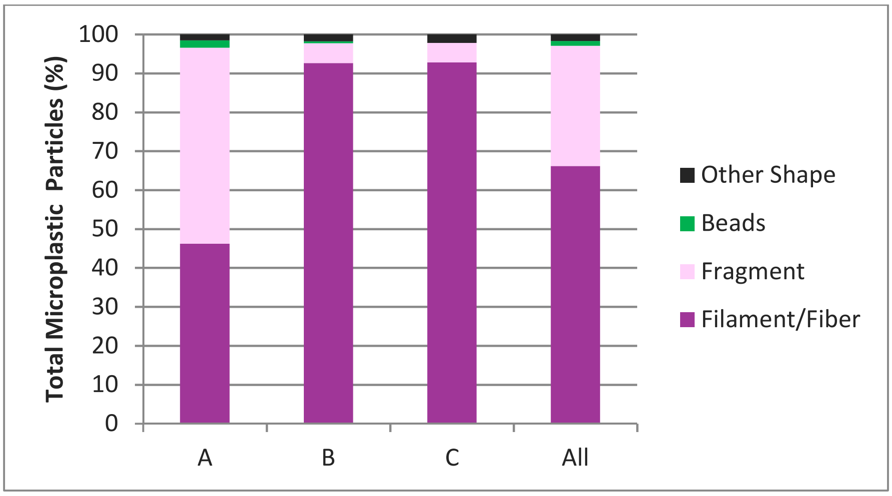

Figure 3-7: Percentage (%) of total microplastic particles in each category in sludge samples during the period from June to October 2018 in the four wastewater treatment plants (Calculation based on calculated microplastic particles per kilo (MP/Kg)).

Regarding the color, 62.2% of overall fibers/filaments were black (MP/Kg- d.w), 16.1% were White/transparent; 9.7% Other color, 6.8% Blue; 4.4% Red and 0.8% were Green (Figure 3-8). At facility A, White/Transparent fragments constituted 89%, Green fragments 5.6%, Other color 3.4% and Blue 2%. No black or red fragments were found in this facility (Figure 3-9).

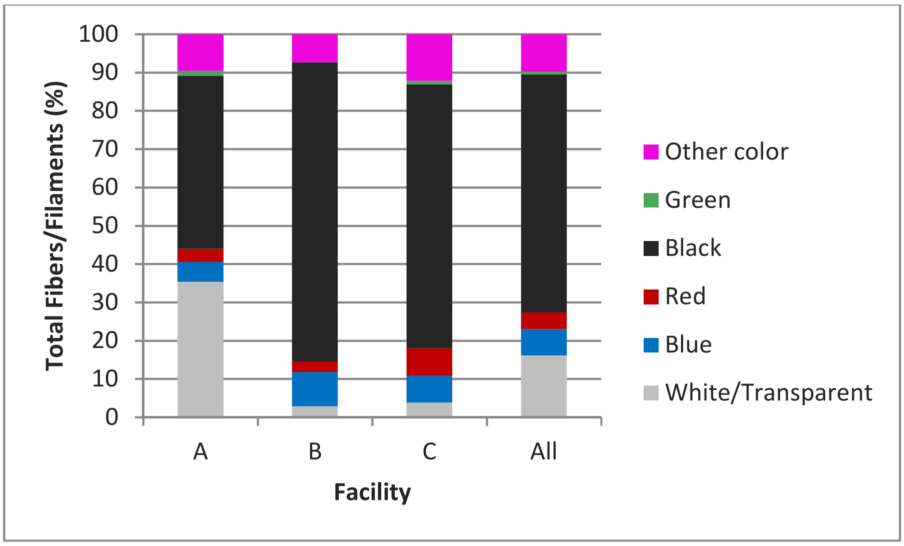

Figure 3-8 Composition of color (%) of Fiber/Filaments in sludge from each facility during the study period from June to October of 2018 (Calculation based on calculated microplastic particles per kilo (MP/Kg –d.w)).

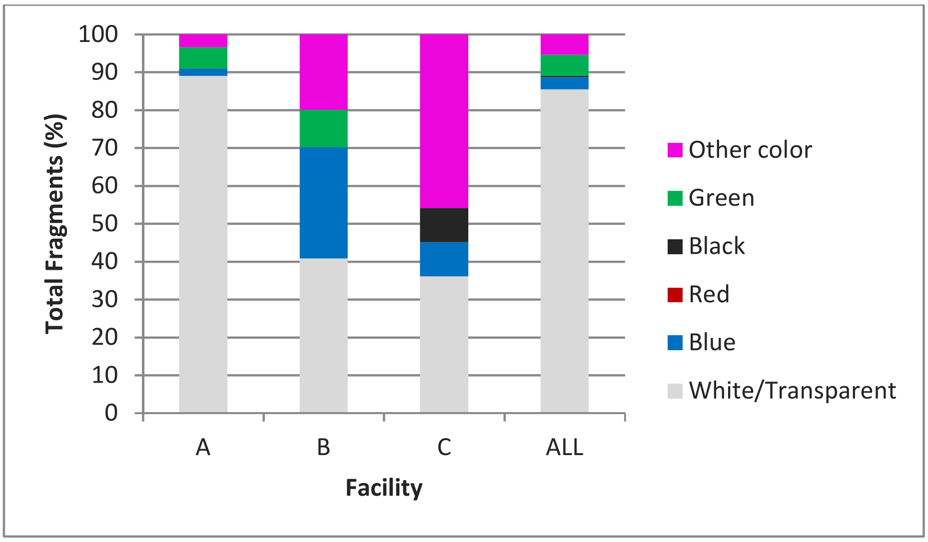

Figure 3-9 Composition of color (%) of Fragments in sludge from each facility during the study period from June to October of 2018 (Percentage based on calculated microplastic particles per kilo (MP/Kg –d.w) data).

3.2.2 Differences between facilities

There was a significant difference between the total microplastic particles in sludge of the three facilities (ANOVA: F value= 18.66; df= 2; p-value: <0.001). However, the difference was only between facility A and the others facilities (Tukey HSD: A-B: p-value <0.001; A-C: p-value <0.001) (Figure 3-10). Facility A had the highest mean when comparing to the others (A= 37502± 10424; B= 13771±2979; C=14419±5417).

There was no significant difference in the total amount of microplastic particles found between the months (ANOVA, F-value= 0.475; df= 4; p-value = 0.753).

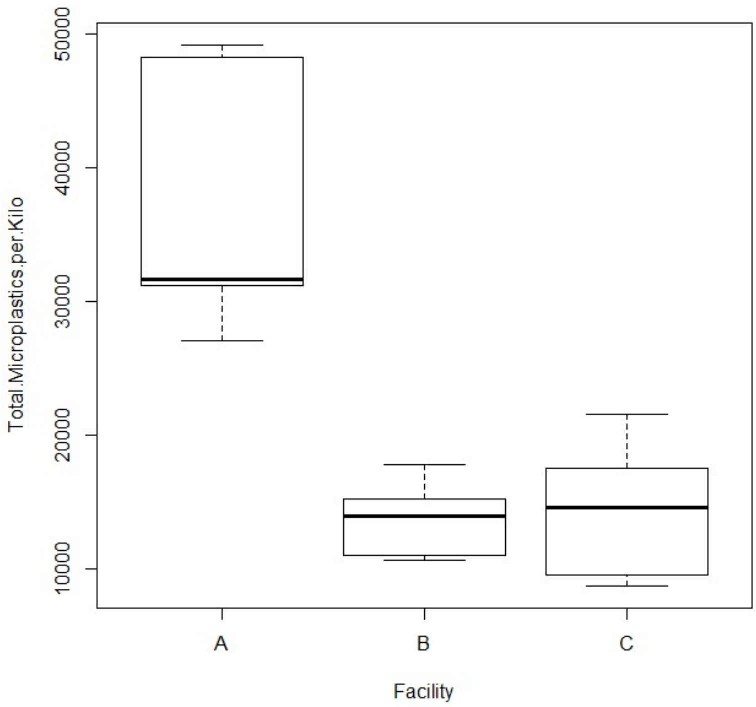

Figure 3-10 Total Amount of microplastic particles per kilo (MP/Kg –d.w) in sludge samples in each wastewater treatment plant during the study period from June to October of 2018.

3.3 Methodology: main observations

3.3.1 Extraction Step

The wastewater procedure generated two filters per sample in some cases (Not included the filter used to clean the remaining content in the cylindrical beaker). It clogs after a certain amount of wastewater. The extraction stage of the microplastic particles was not time consuming. Regarding the pre-treatment with hydrogen peroxide, it eliminate some of the organic matter but not everything. It was still possible to visualize organic matter in the filters. Many filters still had a large amount of cellulose-like fibers.

Regarding the sludge procedure, it was too time consuming during the extraction step. Besides the waiting time of the pre-treatment with Fenton´s regent (2h) and freshwater flotation (1h) and Zinc chloride (2h), the waiting time for the samples to dry in the oven and for the ethanol to evaporate was long. It reached days in many cases.

The Fenton`s reagent degraded a large amount of solid organic matter into liquid solution in a short time, especially in samples of facility A (Treated sludge). In the samples from this facility, the reaction with Fenton was extreme strong when compared to the others. It was possible to visualize a reaction with effective gas bubbles and the production of smoke which did not occur with the other samples.

In facilities B and C, even after the pre-treatment with Fenton, it was possible to visualize a large quantity of solid particles in the sample.

In facility A, all steps of the methodology were implemented. At the facilities B and C, all organic matter raised to the top during the freshwater flotation. It was not possible/useful to proceed with the second flotation step with Zinc chloride.

3.3.2 Analysis Step

The analysis step was difficult and time consuming especially with the wastewater samples. At the sludge samples, the analysis of samples in stereo microscope was less time consuming than wastewater samples.

It was not possible to follow all the criteria mentioned in the methods, as for example: it was almost impossible to poke or compress the fibers to be sure that it was plastic or not; it was not possible or easy to see if the particles were shinny/glassy; it was not possible to meet at least two of the criteria as established by Horton 2017.

The categories of fragments, beads and other shapes were difficult to identify.

3.3.3 Air Contamination Control

The mean of the microplastic particles found in the air control filters was 1.8 microplastic particles per filter (Excluding unknown results). The maximum was 7 microplastic particles in one filter. Fiber/Filaments were the most abundant (Chi-squared goodness of fit test: X-squared: 12; df=1; p-value <0.001). No bead or “other shape” particles were found. Particles included in the unknown had a mean of 3 particles per filter. The maximum of 14 unknown particles were found in one filter.

It is important to emphasize that these particles numbers were after a whole day of work. Several samples were treated in one day, so a single sample was only partly exposed to this air contamination.

4 Discussion

4.1. Wastewater and Sludge samples

4.1.1 Categories and Colors

The overall results showed that, when excluding the unknown particles, fibers/filaments were the most common microplastic particles (MP/L) in the wastewater samples (71.5%). Fragments consisted of 12.8%. In the sludge samples, 93% of the total microplastic particles found in two of three wastewater treatment plants were fibers/filaments. In Overall, Fibers/Filaments corresponded to 66.2% and fragments to 30,9% in the sludge samples.

Similar result were also found by Sutton et al. (2016), who found the dominance of fibers followed by fragments in effluent of eight wastewater treatment plants in San Francisco Bay, California. Large quantity of fibers was also found by Dris et al. (2015) in raw wastewater and by Magnusson and Norén (2014) in influent and sludge samples. Lares et al. (2018) also found a high percentage of fibers in sludge samples in Finland (82%). According to Sun et al. (2019), the average percentage of fibers observed in wastewater is 52.7% and it may be explained by the large amount of fibers that are released through domestic washing machine discharges as shown in some studies (Browne et al. 2011, Napper and Thompson 2016, Pirc et al. 2016). De Falco et al. (2019) reported that depending from the type of washed garment, 640,000 to 1,500,000 microfibers can be released during washing. According to Habib et al. (1998), fibers are released from textiles by mechanical action, during the wash cycles and become suspended in wastewater and incorporated into municipal sewage treatment plant sludge and effluent.

The mean number of fibers/filaments (MP/L) in the facilities varied from 144 (Facility B) to 683 (Facility D) in wastewater influent samples (Table 3-1). In sludge samples (MP/kg-d.w) the mean of fibers were between 12751 (Facility B) and 17329 (Facility A) (Table 3-2). Lusher et al. (2017) found an overall average of 1946 MP/Kg (w.w) and 6077 MP/Kg (d.w) of microplastic particles (not only fibers) in 10 sludge samples investigated from eight wastewater treatment plants in Norway. Mahon et al. (2017) reported a mean abundances of microplastic particles from 4196 to 15385 (MP/Kg – d.w) in sludge samples from seven wastewater treatment plants. However, since these results are based on different methodologies it is difficult to compare.

According to Sutton et al. (2016), some cellulose-derived fibers can survive wet peroxide oxidation (WPO). WPO is a procedure that has been applied to improve the efficiency of sample pre-treatment. It is a liquid phase oxidation process, which is derived from the Fenton`s reaction, using hydrogen peroxide at high temperature and iron salts as catalyte (Debellefontaine et al. 1997). In that way, it is presumed that cellulose may survive treatment by hydrogen peroxide or Fenton`s reaction, which may explain the large amount of cellulose-like fibers found in the samples even after pre-treatment.

Since the samples were not subjected to chemical analysis, only visual identification, it is possible that the presence of non-plastic fibers resulted in an overestimation of the total amount of fibers/filaments. In addition, it was not possible to follow all the criteria, especially for fibers, and this may have contributed to a presence of non- plastic fibers in the results. Sun et al. (2019) mentioned that the visual identification of microplastic particles is open to bias since it depends strongly on the operator and due to the relatively low magnification factor of stereo microscope (size-limited).

According to Sun et al. (2019), some studies also included natural fibers during the quantification. Talvitie et al. (2017) found that 66% of all the textile fibers were natural fibers of cotton, linen or wool. Ladewig et al. (2015) mentioned that natural fibers have been neglected in the studies probably because of the commonly held perception that they are quickly degraded and do not harm the environment. According to him, the natural fibers should have the same environmental concern as synthetic fibers since both have been reported to sorb chemical pollutants.

The global production of textile fibers can be described as 60% synthetic fibers; 30% cotton and 10% other (Carr 2017). Based on this, it may be presumed that a large amount of the fibers in the investigated facilities may be synthetic fibers. In the wastewater samples (MP/L), Black fiber/filaments were most abundant (64.7%). Blue color consisted 14.1%, Red color 8.3%, White/transparent 6.2%, Other Color 5.5% and Green 1.2%. However, White/transparent fibers/filaments may be underestimated due to the high amount of cellulose-like, still present in the filters. Due to this large amount, it was very difficult to distinguishing or see White/transparent fibers. Thereby, white/transparent fibers may have been included as unknown. According to Mintenig et al. (2017), the identification of microplastics based on visual identification can result in overestimation by misidentification but also in an underestimation due to barely visible or transparent particles and fibers. They found that most fibers were categorized as transparent (61%) in treated wastewater. Ziajahromi et al. (2017) found that the identified microplastic fibers were mostly white and transparent along with several shiny green ones in effluent samples of Wastewater treatment plants in Australia.

In the sludge samples, it was somewhat easier to visualize the white/transparent fibers/filaments in samples with dark background and less presence of cellulose-like fibers as occurred in the samples from the facility A. At the other facilities, there were large amounts of cellulose-like fibers that made the identification difficult and the background not so dark. The overall percentage of white/transparent fibers/filaments were lower than black fibers/filaments but higher than the others (Black = 62.2%; white/transparent = 16.1%; other colors = 9.7%; Blue = 6.8% and Red =4.4%).

Regarding the percentage of other categories, fragments was the second most observed category in all samples except for the sludge of facility A. In this facility, the total of fragments was slightly higher than the total of fibers. There was high amount of transparent film-like that may contribute to the high amount of the fragments category. Since there is no film category in this study, it was included in fragments. According to Sun et al. (2019), microplastic film could be mainly from packing products and from the deterioration of plastic bags. This type of particles was not visualized in the other facilities samples.

Beads and other shapes were only found in low amounts in the wastewater and sludge samples. Perhaps this is due to the small size that makes it difficult to identify. According to Lenz et al. (2015), fibers had a higher succes rate than other particles to be identified due to the fact that fibers provide more structural features for identification.

Beads are very small and easily confused with other particles, and thus identification by visual techniques is very challengig. Lusher et al. (2017) reported that 84% of beads found in sludge of wastewater treatment plants in Norway were transparent in colour.

This contributes to the difficult identification. This present study opted for a more conservative analysis in order to try to reduce the misidentification as much as possible. Only beads with perfect spherical shape and bright color were counted. However, Cheung and Fok (2017) found that the majority of the microbead shapes in nine brands of plastic microbeads from facial scrubs were irregular instead of spherical. In this way, The beads could be uderestimated due to complexity of identification of this particles.

These high level of uncertainty also occurred with the identification of particles that might fit into fragments and other shapes category. Particles that could be included in these categories may be framed as unknown. Perhaps, this could contribute to the high number of particles described as unknown.

4.1.2 Difference between facilities

The ANOVA test showed that there was a significant difference in the total amount of microplastic particles between facilities in both wastewater and sludge samples (MP/L and MP/Kg (d.w)). In influent samples, there was significant difference between Facility D and B only.

To analyze why this difference occurs more informations that is needed because there are many variables involved. According to Ou and Zeng (2018), it is difficult to fully clarify the source and composition of microplastic in influent due to knowledge limitations. Wastewater treatment plants receive different sources as domestic wastewater, industrial wastewater, storm and water runoff. Sun et al. (2019) reported that differences of microplastic concentrations between facilities can be related to a complex variety of factors as catchment size, population served, adjacent surrounding land use, wastewater sources, combined sewer systems etc. Also, the preference of residents in the served catchment for wearing synthetic clothes or using plastic products, can directly affect the concentration of microplastics in wastewater (Sun et al. 2019).

No significant correlation between the number of microplastics particles and population equivalents was found by Mintenig et al. (2017) in 12 wastewater treatment plants in Germany. Lusher et al. (2017) also did not find significant correlation between the population equivalent of wastewater treatment plants in Norway and the number of microplastic in sludge. In the present study, no significant correlation was found between the population equivalent of each wastewater treatment plant and the total microplastic found in the influent samples. There was also no significant correlation between the 24h volume flow during the day of sampling and the total number of microplastic particles found in the influent samples.

The mean of total microplastic particles found in each facility had a very high standard deviation value. This may be due to the small sample size (n=5), so the results found should be analyzed with caution.

In the effluent samples, stastical tests showed that the facility D was significant different from the other facilities concerning total microplastic particles. Facility D had the highest amount of total microplastic particles found. According to Sun et al. (2019), the wastewater treatment process applied will affect the microplastic concentration in the effluent. Conley et al. (2019) mentioned that differences in the total amount of microplastics found in the effluent could be explained by differences between facilities as treatment process, flow rate, service population and service composition. More studies are needed to come to some conclusion. Limitations of this study by small sample size and analyzes done with only visual identification, also makes it difficult to obtain a reliable analysis. Sun et al. (2019) mentioned that studies without chemical characterization were very likely to induce bias in quantification, especially distinguishing natural and synthetic fibers.

The stastistical tests showed that there were a significant difference between sludge samples of the Faciliy A and the other facilities (B and C). The facility A had the highest amount of total microplastic particles compared to the others. The sludge from facility A was mixed with the sludge from other facilities during the treatment process which perhps could have affected the result. However, two different methodologies were applied since the flotation step did not work properly for the facilities B and C as will be discussed in chapter 4.2.1 of this study. The samples from the facility A were the ones where the flotation step worked. The differences in methodology may have generated this difference and therefore it is difficult to compare.

4.2 Methodology critical assessment

This critical assessment of the methodology below will be done from the extraction stage and onwards. The sampling stage will not be considered since it was carried out by the staff of each facility.

4.2.1 Pre-treatment and Extraction step

According to Gies et al. (2018), It is a challenge to isolate, enumerate and characterize microplastic particles in an organic-rich wastewater sample due to the diversity in size, structure, color and polymeric composition of microplastic particles.

The wastewater procedure in general worked relatively well, it was not time consuming in the extraction stage of the microplastics but it was time consuming at the analysis step.

Regarding the extraction step, the vacuum filtration step worked well except for the number of filters generated for each sample. Due to the large amount of organic matter, it sometimes clogged and generated two filters per sample (besides the filter used to clean the remaining content in the cylindrical beaker and the glass filter). It produced a larger time analysis in the stereo microscope later on.

In order to try to reduce the organic matter in the filter, approximately seven drops of hydrogen peroxide was added to cover the entire sample. Nuelle et al. (2014) recommended to expose filters to hydrogen peroxide instead of the whole sample because it could be difficult to treat a large amount of sediments. This helped to eliminate some of the organic matter but not satisfactorily. The filters still contained organic matter, especially cellulose-like fibers, which made it difficult to visualize the particles. Perhaps, this dose needs to be better adjusted or other chemical compound should be used. However, Lares et al. (2018) mentioned that although an oxidation treatment was used, substantial amounts of organic fibers and particles were abundant in wastewater and sludge samples. It was also observed in his study, white flat cellulose fibers, mainly originating from toilet papers, together with sand grains and glass fragments were also present in many of the samples.

According to Nuelle et al. (2014) 30% of hydrogen peroxide solution was the ideal reagent for degrading about 50% of biogenic organic matter but some visible changes, as gas bubbles, were observed in some polymers. It indicates that chemical reaction between hydrogen peroxide and the polymers occurred. However, the author did not specify if discoloration is one of the “visible changes” observed with 30% of hydrogen peroxide. The author mentioned only discoloration of particles using 35% of hydrogen peroxide which may complicate the identification rather than facilitate it. According to Zeronian and Inglesby (1995), Peroxides are important bleaching reagents for cellulosic products in industry and it also can degrade cellulose substrate.

In the present study, it was observed that some fibers appeared to be slightly discolored (Mostly black, blue or red fibers). However, it is not possible to confirm that this was due to Hydrogen Peroxide.

Regarding the pre-treatment of sludge samples, peroxidation using 30% hydrogen peroxide is also a common method (Nuelle et al. 2014). However, Fenton`s reagent offers a considerable reduction in sample preparation time (Tagg et al. 2017). According to Lusher et al. (2017), The Fenton´s reagent treatment presented no signs of degradation of microplastics and it also was very good in reducing the proportion of organic material associated with sludge samples.

Unlike the others facilities, a strong reaction occurred when mixing Fenton’s regent with the samples of facility A. This reaction produced effective gas bubbles and smoke. Fenton`s reagent showed to be very helpful since it degraded a large amount of solid organic matter into liquid solution in a short time, especially in the samples of facility A. However, at facilities B and C it did not work so well since even after the pre-treatment with Fenton, it was possible to visualize a large quantity of solid organic matter in the sample. These samples also presented a large amount of cellulose-like fibers. Due to different sludge compositions, perhaps it needs to be better adjusted for each type of sample.

The sludge procedure was too time consuming and since there were different types of sludge it did not work at the flotation step in the samples from facilities B and C. At the flotation step, it was expected that the microplastic would be on the top of the beaker and the organic matter on the bottom. At the facilities B and C, all the organic matter also went to the top. It may be explained by the high amount of cellulose-like fibers present in these samples. According to Lares et al. (2018), cellulose fibers have a density of 1.5 g/cm³ so density separation would not have been appropriate to separate them from microplastics because of the overlapping densities of these polymers. It did not work at the first flotation with “freshwater” either when the density is expected to be less than Zinc Chloride.

The use of two types of flotation is an important step. Lusher et al. (2017) reported that for sludge samples that two density solution step were used, 62% of microplastic particles were extracted with low density solution where 38% were separated out with high density solution. However, 74% of fibers were extracted using high-density solution.

According to Zhao et al. (2018), a fundamental step to extract lighter microplastic from complex matrices containing dense constituents is a density based separation. Concentrated sodium chloride ($NaCl$ 1.2 g/cm³) is reported to be widely used in studies (Hidalgo-Ruz et al. 2012). Sodium Chloride is only effective for polymers with lower density (<1.2 g/cm³) and not suitable for extraction of high density polymers (Claessens et al. 2013). According to Löder and Gerdts (2015), high-density solutions as Zinc chloride are suitable for the extraction of the majority of plastics. They recommended the use of zinc chloride and the recycling of the saturated solution by pressure filtration for financial/environmental reasons.

Another consideration about the sludge procedure is that some filters presented a very thick layer, especially the samples from the facilities where the flotation step did not work to separate particles from organic matter. In this way, it was only possible to check on the top of the thick layer. This may have led to an underestimation of the results.

The quantity of each sample analyzed in the present study was small (10 g w.w). Some studies (Magnusson and Norén 2014, Lusher et al. 2017) also used low quantity of sample. This may be due to the high complexity of the sample and/or the high cost and quantity of high density salts required. Nuelle et al. (2014) mentioned that increasing the sample volume increases the guarantees that random samples are representative of a study area. Also, it increases the prospects of detecting microplastics in areas where the distribution of microplastics is very low or heterogeneous as is the case in some areas for sediments sample.

4.2.2 Analysis step

Regarding the analysis step of wastewater, it was extremely time consuming for many reasons: 1- The large amount of very small particles in each sample; 2- The presence of organic matter; 3- the non-existence of a robust manual for morphological identification of microplastics in wastewater/sludge matrix 4- to visualize small particles, higher magnification is required. In that way, a small area of the filter is analyzed each time; 5- The wide variety of particle types in each sample; 6- the different types of samples and background color; 7- the lack of experience of the researcher in microplastic identification.

According to Song et al. (2015), the smaller the size of microplastic particles the more difficult to identify. The ambiguous characteristics of non-plastics (resembling plastics) and plastics (resembling non-plastics) make this identification difficult.

Regarding the criteria to identify microplastics, it was not possible to follow all the criteria. Criteria like shiny/glassy by example was not possible to visualize in all particles, especially fibers. This could be due to the small size of these particles and also the interference of light from stereo microscope, making recognition difficult through visual identification. According to Conley et al. (2019), during chemical digestion, there is a potential for damage and degradation of fibers, so shine and tapering are not appropriate criteria for studies that use this method. In the present study, It was not possible to poke the fibers or other particles with hot needle due to the very small size of particles. The statement of Horton et al. (2017) that particles should meet at least two of the criteria described by them was not possible to use since many likely plastic would not be considered.

In wastewater samples, 50 areas of the filters were chosen to be analyzed. Perhaps it is better to use time limit to analyze each filter instead of a specific quantity of areas so the time used for the analysis will be lower. Although, when you do not use a specific time for each filter, it is presumed a better work quality. This methodology may also lead to an overestimation or underestimation in the results since the value observed in each filter (direct counted particle) is estimated for the total area of the filter used for filtration, assuming that the particles will have the same frequency in the rest of filter.

The analysis of the sludge samples was faster than the wastewater samples. In the sludge samples, the whole filter was visualized for maximum 15 minutes. This method worked fine for this type of sample since it was not so time consuming as the wastewater method and there is no estimation of the particles found in the filter. It was also possible to view the entire filter. The result seems to be more accurate than the wastewater methodology.

Due to financial limits, analyses of microplastic in the wastewater and sludge samples was based only in visual identification. In this study, the size of the particles was not measured. According to Lusher et al. (2017), 81% of the amount of microplastics found in the sludge samples were below 1mm, with an average size of 644 µm and 34% of plastics particles had a size between 50 and 125 µm. Mintenig et al. (2017) found that around 59% of microplastic particles had a size between 50 and 100 µm in wastewater effluent. This very small size makes visual identification an enormous challenge.

According to Song et al. (2015), identification using microscope method is easier and faster than FT-IR. However, microplastics below 1mm in size are more likely to be missed or miscounted. The misidentification rate of visual identification showed in previous studies varies from 20% to 70% (Hidalgo-Ruz et al. 2012, Eriksen et al. 2013, Song et al. 2015). In this manner, the use of a second method as chemical analysis is highly recommended.

4.2.3 Air contamination control

The results showed that most of the particles found in the air control were fibers. This result is in accordance with Dris et al. (2015), who found fibers in atmospheric fallout in a percentage of 90% of the total microplastics observed.

According to them, airborne fibers are the main cause of contamination problems in Labs, since it is easily transported by air and it is omnipresent in the environment.

In this context, although the results showed a low mean per filter, it is important to take preventive measures to avoid contamination of samples. Whenever possible the samples were covered with aluminum foil in order to try to reduce any contamination by air.

5 Conclusion

Fibers/Filaments was the most common category of microplastic particles found in the four wastewater treatment plants, in both wastewater and sludge samples. The only exception was the sludge samples of one facility, where fragments had higher percentage than fibers/filaments. Regarding the colour, black fibers/filaments were more common than the other colours. However, white/transparent fibers may be underestimated due to the large amount of cellulose-like fibers found in the samples. There was a significant difference in the total number of microplastic particles found between some facilities in wastewater and sludge samples. However, these results should be analysed with caution. It is possible that the presence of non-plastic particles resulted in an overestimation of the results. Underestimation of microplastic particles could also be the case due to barely visible particles and the difficult identification of so small particles since the analysis was performed only by visual identification. To get to a solid conclusion the use of a second method of identification such as chemical analysis is required.

Microplastics is a relative new topic and there is no standardized methodology so far. To find a methodology that better fits to the samples is a challenge. In that way, many steps in the methodology used in this study were discussed to contribute with information to future studies on the development of an ideal methodology in this field.

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Annex 1 - Wastewater samples data

Facility A: Influent data

1- Directly counted (D.C) during visual identification = Rough data

A Influent total – D.C						
Month (all filter)	Filament/Fiber	Fragments	Beads	Other Shape	Total	Unknown
June	13	0	0	0	13	76
July	24	1	0	1	26	40
August	49	6	0	0	55	39
September	18	0	2	2	22	140
October	48	0	1	0	49	16
Total	152	7	3	3	165	311

A Influent 50 areas only (Rough data)						
Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	2	0	0	0	2	72
July	3	1	0	1	5	37
August	4	0	0	0	4	29
September	5	0	2	2	9	139
October	4	0	1	0	5	13
Total	18	1	3	3	25	290

A Influent Overlook /clean (Rough data)						
Month	Filament/fiber	Fragment	Beads	Other Shape	Total	Unknown
June	11	0	0	0	11	4
July	21	0	0	0	21	3
August	45	6	0	0	51	10
September	13	0	0	0	13	1
October	44	0	0	0	44	3
Total	134	6	0	0	140	21

1- Calculated particles

A Influent 50 area only		with formula				
Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	6,94	0	0	0	6,94	249,84
July	10,41	3,47	0	3,47	17,35	128,39
August	13,88	0	0	0	13,88	100,63
September	17,35	0	6,94	6,94	31,23	482,33
October	13,88	0	3,47	0	17,35	45,11
Total	62,46	3,47	10,41	10,41	86,75	1006,3

A Influent Overlook / clean						
Month	Filament	Fragment	Beads	Other shape	Total	Unknown
June	11	0	0	0	11	4
July	21	0	0	0	21	3
August	45	6	0	0	51	10
September	13	0	0	0	13	1
October	44	0	0	0	44	3
Total	134	6	0	0	140	21

A Total after formula		in 100 ml	With June fixed			
Month	Filament	Fragment	Beads	Other shape	**Total**	**Unknown**
June	13,5	0,0	0,0	0,0	13,5	190,4
July	31,4	3,5	0,0	3,5	38,4	131,4
August	58,9	6,0	0,0	0,0	64,9	110,6
September	30,4	0,0	6,9	6,9	44,2	483,3
October	57,9	0,0	3,5	0,0	61,4	48,1
Total	192,0	9,5	10,4	10,4	222,3	963,8

A Total after formula		in 1L				
Month/type	Filament	Fragment	Beads	Other shape	Total	Unknown
June	134,6	0,0	0,0	0,0	134,6	1903,8
July	314,1	34,7	0,0	34,7	383,5	1313,9
August	588,8	60,0	0,0	0,0	648,8	1106,3
September	303,5	0,0	69,4	69,4	442,3	4833,3
October	578,8	0,0	34,7	0,0	613,5	481,1
Total	1919,8	94,7	104,1	104,1	2222,7	9638,4

Facility A: Effluent data

1- Directly counted (D.C) during visual identification = Rough data

A Effluent total D.C - Rough data						
Month (all filter)	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	14	3	1	1	19	140
July	11	1	0	1	13	42
August	21	0	0	0	21	38
September	12	1	3	5	21	241
October	18	0	1	1	20	42
Total	76	5	5	8	94	503

A Effluent 50 area only						
Month/type	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	2	2	1	1	6	140
July	0	0	0	1	1	40
August	1	0	0	0	1	36
September	3	1	3	5	12	235
October	1	0	1	1	3	40
Total	7	3	5	8	23	491

A Effluent Overlook and clean						
Month	Filament	Fragment	Beads	Other Shape	Total	Unknown
June	12	1	0	0	13	0
July	11	1	0	0	12	2
August	20	0	0	0	20	2
September	9	0	0	0	9	6
October	17	0	0	0	17	2
Total	69	2	0	0	71	12

1- Calculated particles

A :Effluent - 50 areas only with formula						
Month/type	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	6,94	6,94	3,47	3,47	20,82	485,8
July	0	0	0	3,47	3,47	138,8
August	3,47	0	0	0	3,47	124,92
September	10,41	3,47	10,41	17,35	41,64	815,45
October	3,47	0	3,47	3,47	10,41	138,8
Total	24,29	10,41	17,35	27,76	79,81	1703,77

A : Effluent overlook /clean						
Month/type	Filament	Fragment	Beads	Other shape	Total	Unknown
June	12	1	0	0	13	0
July	11	1	0	0	12	2
August	20	0	0	0	20	2
September	9	0	0	0	9	6
October	17	0	0	0	17	2
Total	69	2	0	0	71	12

A : Total after formula In 100 ML June fixed							
Month/type	Filament	Fragment	Beads	Other shape	**Total**	**Unknown**	
June	14,2	6,0	2,6	2,6	25,4	364,4	100ml
July	11,0	1,0	0,0	3,5	15,5	140,8	
August	23,5	0,0	0,0	0,0	23,5	126,9	
September	19,4	3,5	10,4	17,4	50,6	821,5	
October	20,5	0,0	3,5	3,5	27,4	140,8	
Total	88,6	10,4	16,5	26,9	142,4	1594,3	

A: Total after formula in 1L						
Month/type	Filament	Fragment	Beads	Other shape	Total	Unknown
June	142,1	59,6	26,0	26,0	253,7	3643,6
July	110,0	10,0	0,0	34,7	154,7	1408,0
August	234,7	0,0	0,0	0,0	234,7	1269,2
September	194,1	34,7	104,1	173,5	506,4	8214,5
October	204,7	0,0	34,7	34,7	274,1	1408,0
total	885,6	104,3	164,8	268,9	1423,6	15943,3

Facility B: Influent data

Directly counted (D.C) during visual identification = Rough data.

B Influent Total						
Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	5	2	0	3	10	46
July	18	5	0	0	23	122
August	15	3	1	1	20	87
September	20	4	0	0	24	91
October	4	6	0	3	13	67
Total	62	20	1	7	90	413

B Influent 50 areas only						
Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	2	2	0	3	7	33
July	1	2	0	0	3	115
August	0	3	1	0	4	77
September	0	3	0	0	3	87
October	1	3	0	1	5	59
Total	4	13	1	4	22	371

B influent overlook and clean						
Month	Filament/fiber	Fragment	Beads	Other Shape	Total	Unknown
June	3	0	0	0	3	13
July	17	3	0	0	0	7
August	15	0	0	1	16	10
September	20	1	0	0	21	4
October	3	3	0	2	0	8
Total	58	7	0	3	40	42

1- Calculated particles

B influent 50 area only		with formula				
Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	6,94	6,94	0	10,41	24,29	114,51
July	3,47	6,94	0	0	10,41	399,05
August	0	10,41	3,47	0	13,88	267,19
September	0	10,41	0	0	10,41	301,89
October	3,47	10,41	0	3,47	17,35	204,73
Total	13,88	45,11	3,47	13,88	76,34	1287,37

B influent overlook and clean filter						
Month	Filament	Fragment	Beads	Other shape	Total	Unknown
June	3	0	0	0	3	13
July	17	3	0	0	20	7
August	15	0	0	1	16	10
September	20	1	0	0	21	4
October	3	3	0	2	8	8
Total	58	7	0	3	68	42

B Total after formula		In 100 ml				
Month	Filament	Fragment	Beads	Other shape	Total	Unknown
June	9,9	6,9	0,0	10,4	27,3	127,5
July	20,5	9,9	0,0	0,0	30,4	406,1
August	15,0	10,4	3,5	1,0	29,9	277,2
September	20,0	11,4	0,0	0,0	31,4	305,9
October	6,5	13,4	0,0	5,5	25,4	212,7
Total	71,9	52,1	3,5	16,9	144,3	1329,4

B Total after formula		In 1L				
Month	Filament	Fragment	Beads	Other shape	Total	Unknown
June	99,4	69,4	0,0	104,1	272,9	1275,1
July	204,7	99,4	0,0	0,0	304,1	4060,5
August	150,0	104,1	34,7	10,0	298,8	2771,9
September	200,0	114,1	0,0	0,0	314,1	3058,9
October	64,7	134,1	0,0	54,7	253,5	2127,3
Total	718,8	521,1	34,7	168,8	1443,4	13293,7

Facility B: Effluent data

Directly counted (D.C) during visual identification = Rough data.

B Effluent Total						
Month	Filament/Fiber	Frag.	Beads	Other Shape	Total	Unkn.
June	6	5	0	0	11	42
July	9	0	1	0	10	28
August	5	3	0	0	8	27
September	7	2	0	2	11	52
October	5	0	0	0	5	44
Total	32	10	1	2	45	193

B Effluent 50 areas only						
Month	Filament/Fiber	Frag.	Beads	Other Shape	Total	Unkn.
June	1	3	0	0	4	38
July	2	0	0	0	2	21
August	0	1	0	0	1	27
September	0	2	0	2	4	46
October	0	0	0	0	0	40
Total	3	6	0	2	11	172

B Effluent Overlook and clean						
Month	Filament/fibers	Frag.	Beads	Other Shape	Total	Unkn.
June	5	2	0	0	7	4
July	7	0	1	0	0	7
August	5	2	0	0	7	0
September	7	0	0	0	7	6
October	5	0	0	0	0	4
Total	29	4	1	0	21	21

1- Calculated particles

B Effluent 50 area only	with formula					
Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	3,47	10,41	0	0	13,88	131,86
July	6,94	0	0	0	6,94	72,87
August	0	3,47	0	0	3,47	93,69
September	0	6,94	0	6,94	13,88	159,62
October	0	0	0	0	0	138,8
Total	10,41	20,82	0	6,94	38,17	596,84

B Effluent Overlook/clean						
Month	Filament	Fragment	Beads	Other shape	Total	Unknown
June	5	2	0	0	7	4
July	7	0	1	0	8	7
August	5	2	0	0	7	0
September	7	0	0	0	7	6
October	5	0	0	0	5	4
Total	29	4	1	0	34	21

B Total after formula	In 100 ml					
Month	Filament	Fragment	Beads	Other shape	Total	Unknown
June	8,5	12,4	0,0	0,0	20,9	135,9
July	13,9	0,0	1,0	0,0	14,9	79,9
August	5,0	5,5	0,0	0,0	10,5	93,7
September	7,0	6,9	0,0	6,9	20,9	165,6
October	5,0	0,0	0,0	0,0	5,0	142,8
Total	39,4	24,8	1,0	6,9	72,2	617,8

B Total after formula	In 1L					
Month/type	Filament	Fragment	Beads	Other shape	Total	Unknown
June	84,7	124,1	0,0	0,0	208,8	1358,6
July	139,4	0,0	10,0	0,0	149,4	798,7
August	50,0	54,7	0,0	0,0	104,7	936,9
September	70,0	69,4	0,0	69,4	208,8	1656,2
October	50,0	0,0	0,0	0,0	50,0	1428,0
Total	394,1	248,2	10,0	69,4	721,7	6178,4

Facility C: Influent data

Directly counted (D.C) during visual identification = Rough data.

C Infl Total	(Rough data)					
Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	14	4	4	1	23	64
July	31	6	2	1	40	68
August	8	4	0	2	14	62
September	21	1	3	2	27	93
October	30	7	0	3	40	195
Total	104	22	9	9	144	482

C Influent 50 area only						
Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	2	4	4	0	10	60
July	4	1	2	0	7	53
August	4	4	0	2	10	54
September	8	1	3	2	14	88
October	1	3	0	3	7	186
Total	0	13	9	7	48	441

C Influent Overlook and clean						
Month	Filament	Fragment	Beads	Other Shape	Total	Unknown
June	12	0	0	1	13	4
July	27	5	0	1	33	15
August	4	0	0	0	4	8
September	13	0	0	0	13	5
October	29	4	0	0	33	9
Total	85	9	0	2	96	41

1- Calculated particles

C influent 50 area only	with formula					
Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	6,94	13,88	13,88	0	34,7	208,2
July	13,88	3,47	6,94	0	24,29	183,91
August	13,88	13,88	0	6,94	34,7	187,38
September	27,76	3,47	10,41	6,94	48,58	305,36
October	3,47	10,41	0	10,41	24,29	645,42
Total	65,93	45,11	31,23	24,29	166,56	1530,27

C influent Overlook and clean						
Month	Filament	Fragment	Beads	Other shape	Total	Unknown
June	12	0	0	1	13	4
July	27	5	0	1	33	15
August	4	0	0	0	4	8
September	13	0	0	0	13	5
October	29	4	0	0	33	9
Total	85	9	0	2	96	41

C influent Total after formula	In 100 ml					
Month	Filament	Fragment	Beads	Other shape	Total	Unknown
June	18,94	13,88	13,88	1	47,7	212,2
July	40,88	8,47	6,94	1	57,29	198,91
August	17,88	13,88	0	6,94	38,7	195,38
September	40,76	3,47	10,41	6,94	61,58	310,36
October	32,47	14,41	0	10,41	57,29	654,42
Total	150,93	54,11	31,23	26,29	262,56	1571,27

C influent Total after formula	In 1L					
Month/type	Filament	Fragment	Beads	Other shape	Total	Unknown
June	189,4	138,8	138,8	10	477	2122
July	408,8	84,7	69,4	10	572,9	1989,1
August	178,8	138,8	0	69,4	387	1953,8
September	407,6	34,7	104,1	69,4	615,8	3103,6
October	324,7	144,1	0	104,1	572,9	6544,2
Total	1509,3	541,1	312,3	262,9	2625,6	15712,7

Facility C: Effluent data

Directly counted (D.C) during visual identification = Rough data.

C Effluent Total (Rough data)						
Month	Filament/Fiber	Fragmt	Beads	Other Shape	Total	Unknown
June	11	0	0	0	11	37
July	9	1	0	1	11	64
August	7	3	0	1	11	90
September	2	0	1	2	5	169
October	9	1	3	0	13	26
Total	38	5	4	4	51	386

C Effluent 50 area only						
Month	Filament/Fiber	Fragmt	Beads	Other Shape	Total	Unknown
June	2	0	0	0	2	36
July	1	0	0	1	2	64
August	2	2	0	1	5	87
September	1	0	1	2	4	165
October	2	1	3	0	6	24
Total	8	3	4	4	19	376

C Effluent Overlook and clean						
Month	Filament	Fragmt	Beads	Other Shape	Total	Unknown
June	9	0	0	0	9	1
July	8	1	0	0	0	0
August	5	1	0	0	6	3
September	1	0	0	0	1	4
October	7	0	0	0	0	2
Total	30	2	0	0	16	10

1- Calculated particles

C Effluent 50 area only	with formula					
Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	6,94	0	0	0	6,94	124,92
July	3,47	0	0	3,47	6,94	222,08
August	6,94	6,94	0	3,47	17,35	301,89
September	3,47	0	3,47	6,94	13,88	572,55
October	6,94	3,47	10,41	0	20,82	83,28
Total	27,76	10,41	13,88	13,88	65,93	1304,72

C Effluent Overlook/clean						
Month	Filament/Fiber	Fragment	Beads	Other shape	Total	Unknown
June	9	0	0	0	9	1
July	8	1	0	0	9	0
August	5	1	0	0	6	3
September	1	0	0	0	1	4
October	7	0	0	0	7	2
Total	30	2	0	0	32	10

C Effluent Total	after formula		In 100 ml			
Month	Filament/fiber	Fragment	Beads	Other shape	Total	Unknown
June	15,9	0,0	0,0	0,0	15,9	125,9
July	11,5	1,0	0,0	3,5	15,9	222,1
August	11,9	7,9	0,0	3,5	23,4	304,9
September	4,5	0,0	3,5	6,9	14,9	576,6
October	13,9	3,5	10,4	0,0	27,8	85,3
Total	57,8	12,4	13,9	13,9	97,9	1314,7

C Effluent Total	after formula		In 1L			
Month	Filament/fiber	Fragment	Beads	Other shape	Total	Unknown
June	159,4	0,0	0,0	0,0	159,4	1259,2
July	114,7	10,0	0,0	34,7	159,4	2220,8
August	119,4	79,4	0,0	34,7	233,5	3048,9
September	44,7	0,0	34,7	69,4	148,8	5765,5
October	139,4	34,7	104,1	0,0	278,2	852,8
Total	577,6	124,1	138,8	138,8	979,3	13147,2

Facility D: Influent data

Directly counted (D.C) during visual identification = Rough data.

D Infl Total (Rough data)						
Month/type	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	20	1	0	1	22	143
July	31	1	0	2	34	123
August	34	4	0	2	40	92
September	30	2	1	2	35	91
October	130	8	0	2	140	132
Total	245	16	1	9	271	581

D Influent 50 area only (Rough data)						
Month/type	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	13	1	0	1	15	137
July	0	1	0	1	2	101
August	11	3	0	2	16	81
September	10	2	1	2	15	84
October	5	3	0	2	10	108
Total	39	10	1	8	58	511

D infl. Overlook and clean (Rough data)						
Month/type	Filament	Fragment	Beads	Other Shape	Total	Unknown
June	7	0	0	0	7	6
July	31	0	0	1	0	22
August	23	1	0	0	24	11
September	20	0	0	0	20	7
October	125	5	0	0	0	24
Total	206	6	0	1	51	70

1- Calculated particles

D Influent 50 area only	with formula					
Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	45,11	3,47	0	3,47	52,05	475,39
July	0	3,47	0	3,47	6,94	350,47
August	38,17	10,41	0	6,94	55,52	281,07
September	34,7	6,94	3,47	6,94	52,05	291,48
October	17,35	10,41	0	6,94	34,7	374,76
Total	135,33	34,7	3,47	27,76	201,26	1773,17

D Influent Overlook/clean						
Month	Filament	Fragment	Beads	Other shape	Total	Unknown
June	7	0	0	0	7	6
July	31	0	0	1	32	22
August	23	1	0	0	24	11
September	20	0	0	0	20	7
October	125	5	0	0	130	24
Total	206	6	0	1	213	70

D Influent total	after formula			in 100 ml		
Month	Filament	Fragment	Beads	Other shape	Total	Unknown
June	52,1	3,5	0,0	3,5	59,1	481,4
July	31,0	3,5	0,0	4,5	38,9	372,5
August	61,2	11,4	0,0	6,9	79,5	292,1
September	54,7	6,9	3,5	6,9	72,1	298,5
October	142,4	15,4	0,0	6,9	164,7	398,8
Total	341,3	40,7	3,5	28,8	414,3	1843,2

D Influent total	after formula			In 1L		
Month	Filament	Fragment	Beads	Other shape	Total	Unknown
June	521,1	34,7	0,0	34,7	590,5	4813,9
July	310,0	34,7	0,0	44,7	389,4	3724,7
August	611,7	114,1	0,0	69,4	795,2	2920,7
September	547,0	69,4	34,7	69,4	720,5	2984,8
October	1423,5	154,1	0,0	69,4	1647,0	3987,6
Total	3413,3	407,0	34,7	287,6	4142,6	18431,7

Facility D: Effluent data

Directly counted (D.C) during visual identification = Rough data.

D effluent Total		(Rough data)				
Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	3	2	4	5	14	74
July	20	4	5	0	29	180
August	58	0	0	0	58	28
September	84	0	2	0	86	1064
October	89	1	0	2	92	77
Total	254	7	11	7	279	1423

D effluent 50 area only						
Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	0	1	4	5	10	73
July	7	2	5	0	14	176
August	0	0	0	0	0	27
September	5	0	2	0	7	1063
October	4	1	0	2	7	76
Total	16	4	11	7	38	1415

D effl. Overlook and clean						
Month	Filament	Fragment	Beads	Other Shape	Total	Unknown
June	3	1	0	0	4	1
July	13	2	0	0	15	4
August	58	0	0	0	58	1
September	79	0	0	0	79	1
October	85	0	0	0	85	1
Total	238	3	0	0	241	8

1- Calculated particles

D effluent 50 area only	with formula					
Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	0	3,47	13,88	17,35	34,7	253,31
July	24,29	6,94	17,35	0	48,58	610,72
August	0	0	0	0	0	93,69
September	17,35	0	6,94	0	24,29	3688,61
October	13,88	3,47	0	6,94	24,29	263,72
Total	55,52	13,88	38,17	24,29	131,86	4910,05

D effluent	Overlook and clean					
Month	Filament/fiber	Fragment	Beads	Other shape	Total	Unknown
June	3	1	0	0	4	1
July	13	2	0	0	15	4
August	58	0	0	0	58	1
September	79	0	0	0	79	1
October	85	0	0	0	85	1
Total	238	3	0	0	241	8

D effl. Total after formula	In 100 ml					
Month	Filament/fiber	Fragment	Beads	Other shape	Total	Unknown
June	3,0	4,5	13,9	17,4	38,7	254,3
July	37,3	8,9	17,4	0,0	63,6	614,7
August	58,0	0,0	0,0	0,0	58,0	94,7
September	96,4	0,0	6,9	0,0	103,3	3689,6
October	98,9	3,5	0,0	6,9	109,3	264,7
Total	293,5	16,9	38,2	24,3	372,9	4918,1

D effl. Total after formula	In 1L					
Month	Filament/fiber	Fragment	Beads	Other shape	Total	Unknown
June	30,0	44,7	138,8	173,5	387,0	2543,1
July	372,9	89,4	173,5	0,0	635,8	6147,2
August	580,0	0,0	0,0	0,0	580,0	946,9
September	963,5	0,0	69,4	0,0	1032,9	36896,1
October	988,8	34,7	0,0	69,4	1092,9	2647,2
Total	2935,2	168,8	381,7	242,9	3728,6	49180,5

Color Data

Facility A: Influent

Total: Directly counted (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	2	26	9	110	0	5	152
Fragment	2	0	1	0	0	4	7
Beads	3	0	0	0	0	0	3
Other shape	3	0	0	0	0	0	3
Unknown	210	8	2	32	4	55	311
Total	220	34	12	142	4	64	476
Total without unknown	10	26	10	110	0	9	165

50 areas only (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	2	2	0	10	0	4	18

Overlook + clean filter (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	0	24	9	100	0	1	134

1- Calculated data

50 areas only (with formula)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	6,94	6,94	0	34,7	0	13,88	62,46

Overlook + clean filter

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	0	24	9	100	0	1	134

Total after formula and calculations = In 100 ml

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	5,2	23,2	6,8	101,0	0,0	11,2	147,3

Total after formula and calculations = In 1L

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	52,1	232,1	67,5	1010,3	0,0	111,6	1473,5

Facility A: Effluent

1-Total: Directly counted (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	3	3	6	59	0	5	76
Fragment	3	2	0	0	0	0	5
Beads	5	0	0	0	0	0	5
Other shape	7	0	0	0	0	1	8
Unknown	398	0	0	8	2	95	503
Total	416	5	6	67	2	101	597
Total without unknown	18	5	6	59	0	6	94

50 areas only (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	2	0	2	2	0	1	7

Overlook and clean filter (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	1	3	4	57	0	4	69

1- Calculated data

50 area with formula

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	6,94	0	6,94	6,94	0	3,47	24,29

Overlook and clean filter

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	1	3	4	57	0	4	69

Total after formula in 100 ml

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	6,0	2,3	8,2	48,0	0,0	5,6	70,0

Total after formula in 1L

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	59,6	22,5	82,1	479,6	0,0	56,0	699,7

Facility B: Influent

1- Total: Directly counted (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers- Filament	1	24	5	29	2	1	62
Fragment	9	3	0	4	3	1	20
Beads	1	0	0	0	0	0	1
Other shape	4	0	0	0	0	3	7
Unknown	187	12	5	50	2	157	413
Total	202	39	10	83	7	162	503
Total without unknown	15	27	5	33	5	5	90

50 area only (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	1	0	0	1	2	0	4

Overlook and clean filter (rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	0	24	5	28	0	1	58

1- Estimated data

<u>50 area with formula</u>

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers- Filament	3,47	0	0	3,47	6,94	0	13,88

Overlook and clean filter

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers- Filament	0	24	5	28	0	1	58

Total after formula in 100 ml

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers- Filament	3,5	24,0	5,0	31,5	6,9	1,0	71,9

Total after formula in 1L

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers- Filament	34,7	240,0	50,0	314,7	69,4	10,0	718,8

Facility B: Effluent

1- Total: Directly counted (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	2	4	2	18	1	5	32
Fragment	1	0	1	1	0	7	10
Beads	1	0	0	0	0	0	1
Other shape	2	0	0	0	0	0	2
Unknown	68	2	4	34	2	83	193
Total	74	6	7	53	3	95	238
Total without unknown	6	4	3	19	1	12	45

50 areas only (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	1	1	1	0	0	1	4

Overlook and clean filter (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	1	3	1	18	1	4	28

1- Calculated data

50 areas with formula

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	3,47	3,47	3,47	0	0	3,47	13,88

Overlook and clean filter

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	1	3	1	18	1	4	28

Total after formula in 100 ml

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	4,5	6,5	4,5	18,0	1,0	7,5	41,9

Total after formula in 1L

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	44,7	64,7	44,7	180,0	10,0	74,7	418,8

Facility C: Influent

1- Total: Directly counted (Rough data)

Shape	White/Transparet	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	5	23	11	58	0	7	104
Fragment	6	6	1	1	0	8	22
Beads	9	0	0	0	0	0	9
Other shape	7	1	0	0	0	1	9
Unknown	317	28	4	13	18	102	482
Total	344	58	16	72	18	118	626
Total without unknown	27	30	12	59	0	16	144

50 areas only (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	4	4	2	6	0	3	19

Overlook and clean filter (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	1	19	9	52	0	4	85

1- Calculated data

50 areas with formula

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	13,88	13,88	6,94	20,82	0	10,41	65,93

Overlook and clean filter

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	1	19	9	52	0	4	85

Total after formula in 100 ml

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	14,9	32,9	15,9	72,8	0,0	14,4	150,9

Total after formula in 1L

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	148,8	328,8	159,4	728,2	0,0	144,1	1509,3

Facility C: Effluent

1- Total: Directly counted (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers- Filament	3	5	2	27	0	1	38
Fragment	1	2	0	2	0	0	5
Beads	4	0	0	0	0	0	4
Other shape	4	0	0	0	0	0	4
Unknown	323	1	4	6	0	52	386
Total	335	8	6	35	0	53	437
Total without unknown	12	7	2	29	0	1	51

50 areas only (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers- Filament	3	2	0	4	0	0	9

Overlook and clean filter (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers- Filament	0	3	2	23	0	1	29

1- Calculated data

50 areas with formula

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	10,41	6,94	0	13,88	0	0	31,23

Overlook and clean filter

Shape	White/Transarent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	0	3	2	23	0	1	29

Total after formula in 100 ml

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	10,4	9,9	2,0	36,9	0,0	1,0	60,2

Total after formula in 1L

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	104,1	99,4	20,0	368,8	0,0	10,0	602,3

Facility D: Influent

1- Total: Directly counted (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	3	30	24	170	6	12	245
Fragment	6	3	0	0	0	7	16
Beads	1	0	0	0	0	0	1
Other shape	5	0	0	1	0	3	9
Unknown	247	103	7	47	38	139	581
Total	262	136	31	218	44	161	852
Total without unknown	15	33	24	171	6	22	271

50 areas only (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	2	9	5	23	0	1	40

Overlook and clean filter (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	1	21	19	147	6	11	205

1- Calculated data

50 areas with formula

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filaments	6,9	31,2	17,4	79,8	0,0	3,5	138,8

Overlook and clean filter

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filaments	1,0	21,0	19,0	147,0	6,0	11,0	205,0

Total after formula in 100 ml

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filaments	7,9	52,2	36,4	226,8	6,0	14,5	343,8

Total after formula in 1L

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filaments	79,4	522,3	363,5	2268,1	60,0	144,7	3438,0

Facility D: Effluent

1- Total: Directly counted (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers- Filament	8	8	18	216	0	4	254
Fragment	2	2	1	0	0	2	7
Beads	4	0	0	0	0	7	11
Other shape	7	0	0	0	0	0	7
Unknown	1301	4	1	7	0	110	1423
Total	1322	14	20	223	0	123	1702
Total without unknown	21	10	19	216	0	13	279

50 areas only (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers- Filament	5	2	0	1	0	2	10

Overlook and clean filter (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers- Filament	3	6	18	215	0	2	244

1- Calculated data

50 areas with formula

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	17,35	6,94	0	3,47	0	6,94	34,7

Overlook and clean filter

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	3	6	18	215	0	2	244

Total after formula in 100 ml

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	20,4	12,9	18,0	218,5	0,0	8,9	278,7

Total after formula in 1L

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	203,5	129,4	180,0	2184,7	0,0	89,4	2787,0

Annex 2- Sludge data

Facility A

Directly counted particles (Rough data) - d.w

Month/type	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June (3,62g)	50	56	7	0	113	147
July (3,60g)	45	66	1	2	114	91
August (3,76g)	42	58	1	1	102	81
September (3,79g)	101	74	4	4	183	193
October (3,82g)	86	98	0	4	188	152
Total	324	352	13	11	700	664

Particles per Kilo (MP/Kg) –d.w

Month/type	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	13812,2	15469,6	1933,7	0,0	31215,5	40607,7
July	12500,0	18333,3	277,8	555,6	31666,7	25277,8
August	11170,2	15425,5	266,0	266,0	27127,7	21542,6
September	26649,1	19525,1	1055,4	1055,4	48285,0	50923,5
October	22513,1	25654,5	0,0	1047,1	49214,7	39790,6
Total	86644,5	94408,0	3532,8	2924,0	187509,4	178142,1

By Color

Directly counted particles (Rough data)

Shape	White/Transparent	Blue	Red	black	Green	Other colors	Total
Fibers- Filament	115	17	11	146	4	31	324
Fragment	313	7	0	0	20	12	352
Beads	12	0	0	1	0	0	13
Other shape	6	0	1	3	0	1	11
Unknown	442	1	0	60	10	151	664
Total	888	25	12	210	34	195	1364
Total without unknown	446	24	12	150	24	44	700

Fibers (MP/Kg) (d.w)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
June	4143,6	276,2	552,5	7182,3	0,0	1657,5	13812,2
July	3055,6	277,8	555,6	6944,4	0,0	1666,7	12500,0
August	3989,4	531,9	531,9	5319,1	0,0	797,9	11170,2
September	9762,5	1847,0	791,6	11081,8	791,6	2374,7	26649,1
October	9685,9	1570,7	523,6	8638,7	261,8	1832,5	22513,1
Total	30637,0	4503,6	2955,1	39166,5	1053,3	8329,1	86644,5

FRAGMENTS (MP/Kg- d.w)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
June	14917,1	0,0	0,0	0,0	552,5	0,0	15469,6
July	17222,2	555,6	0,0	0,0	277,8	277,8	18333,3
August	13297,9	266,0	0,0	0,0	797,9	1063,8	15425,5
September	15831,1	791,6	0,0	0,0	1847,0	1055,4	19525,1
October	22774,9	261,8	0,0	0,0	1832,5	785,3	25654,5
Total	84043,2	1874,8	0,0	0,0	5307,6	3182,4	94408,0

Facility B

Directly counted particles (Rough data)

Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	31	2	0	0	33	51
July	54	1	0	0	55	44
August	28	1	0	0	29	40
September	33	1	0	3	37	93
October	34	5	1	0	40	95
Total	180	10	1	3	194	323

Microplastic Particles per Kilo (MP/Kg – d.w)

Month	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June (3,08g)	10064,9	649,4	0,0	0,0	10714,3	16558,4
July (3,08g)	17532,5	324,7	0,0	0,0	17857,1	14285,7
August (2,62g)	10687,0	381,7	0,0	0,0	11068,7	15267,2
September (2,43g)	13580,2	411,5	0,0	1234,6	15226,3	38271,6
October (2,86g)	11888,1	1748,3	349,7	0,0	13986,0	33216,8
Total	63752,8	3515,5	349,7	1234,6	68852,5	117599,7

By Color

Directly counted microplastic particles (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other color	Total
Fibers-Filament	5	16	5	141	0	13	180
Fragment	4	3	0	0	1	2	10
Beads	1	0	0	0	0	0	1
Other shape	1	0	0	0	0	2	3
Unknown	80	15	3	69	6	150	323
Total	91	34	8	210	7	167	517
Total without unknown	11	19	5	141	1	17	194

Fiber (MP/Kg –d.w)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
June	649,4	649,4	324,7	7792,2	0,0	649,4	10064,9
July	0,0	1298,7	649,4	14610,4	0,0	974,0	17532,5
August	763,4	1145,0	381,7	7633,6	0,0	763,4	10687,0
September	411,5	823,0	411,5	10699,6	0,0	1234,6	13580,2
October	0,0	1748,3	0,0	9090,9	0,0	1049,0	11888,1
Total	1824,2	5664,4	1767,2	49826,7	0,0	4670,3	63752,8

Fragments (MP/Kg –d.w)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
June	0,0	649,4	0,0	0,0	0,0	0,0	649,4
July	324,7	0,0	0,0	0,0	0,0	0,0	324,7
August	0,0	381,7	0,0	0,0	0,0	0,0	381,7
September	411,5	0,0	0,0	0,0	0,0	0,0	411,5
October	699,3	0,0	0,0	0,0	349,7	699,3	1748,3
Total	1435,5	1031,0	0,0	0,0	349,7	699,3	3515,5

Facility C

Directly counted microplastic particles (Rough data) – d.w

Month/type	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June (3,04g)	25	4	0	0	29	65
July (2,39g)	35	0	0	0	35	20
August (2,74g)	22	1	0	1	24	29
September (3,25g)	51	3	0	3	57	58
October (3,1g)	63	3	0	1	67	101
Total	196	11	0	5	212	273

Particles per Kilo (MP/Kg – d.w)

Month/type	Filament/Fiber	Fragment	Beads	Other Shape	Total	Unknown
June	8223,7	1315,8	0,0	0,0	9539,5	21381,6
July	14644,4	0,0	0,0	0,0	14644,4	8368,2
August	8029,2	365,0	0,0	365,0	8759,1	10583,9
September	15692,3	923,1	0,0	923,1	17538,5	17846,2
October	20322,6	967,7	0,0	322,6	21612,9	32580,6
Total	66912,1	3571,6	0,0	1610,6	72094,3	90760,5

By Color

Directly counted particles (Rough data)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
Fibers-Filament	8	14	15	133	2	24	196
Fragment	4	1	0	1	0	5	11
Beads	0	0	0	0	0	0	0
Other shape	2	0	0	0	0	3	5
Unknown	86	14	12	48	14	99	273
Total	100	29	27	182	16	131	485
Total without unknown	14	15	15	134	2	32	212

Fiber (MP/Kg –d.w)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
June	1315,8	328,9	0,0	4934,2	0,0	1644,7	8223,7
July	0,0	836,8	418,4	12133,9	0,0	1255,2	14644,4
August	0,0	0,0	0,0	6934,3	0,0	1094,9	8029,2
September	307,7	1846,2	2153,8	10153,8	307,7	923,1	15692,3
October	967,7	1612,9	2258,1	11935,5	322,6	3225,8	20322,6
Total	2591,2	4624,8	4830,3	46091,7	630,3	8143,7	66912,1

Fragments (MP/Kg –d.w)

Shape	White/Transparent	Blue	Red	Black	Green	Other colors	Total
june	657,9	0,0	0,0	0,0	0,0	657,9	1315,8
July	0,0	0,0	0,0	0,0	0,0	0,0	0,0
August	0,0	0,0	0,0	0,0	0,0	365,0	365,0
September	307,7	0,0	0,0	0,0	0,0	615,4	923,1
October	322,6	322,6	0,0	322,6	0,0	0,0	967,7
Total	1288,2	322,6	0,0	322,6	0,0	1638,2	3571,6

Annex 3 - 24h volume flow data during the sampling

Facility	H wastewater cubic flow M^3/d
A	16 164
A	16 835
A	14 336
A	17 817
A	14 428
B	1 800
B	2 832
B	3 100
B	5 143
B	3 325
C	4 150
C	3 010
C	3 690
C	4 870
C	4 110
D	2 768
D	2 492
D	2 559
D	3 470
D	2 438

Annex 4 Air contamination control data

Date	Place	Fiber/Filament	Fragment	Unknown
26.10	Place 1	0	3	7
	Place 2	3	4	3
	Place 3	3	1	5
30.10	Place 1	0	0	2
	Place 2	0	0	1
	Place 3	1	1	5
20.11	Work Table	2	0	1
	Fume Hood	2	0	3
30.11	Work Table	2	0	1
	Fume Hood	3	0	1
4.12	Work Table	1	0	6
	Fume Hood	1	0	5
5.12	Work Table	1	1	1
	Fume Hood	1	0	1
6.12	Work Table	0	1	0
	Fume Hood	2	1	1
8.12	Work Table	2	0	4
	Fume Hood	0	0	2
9.12	Work Table	1	0	1
	Fume Hood	1	0	0
13.12	Work Table	1	0	0
	Fume Hood	5	0	2
9.02	Work Table	0	0	10
11.02	Work Table	0	0	0
	Fume Hood	0	0	1
14.02	Work Table	3	0	14
	Fume Hood	1	0	3